Ophthalmic Sensor

The invention is related to an ophthalmic sensor which comprises an ophthalmic device having a polymer matrix and a molecular sensing moiety which interacts with sugar to provide an optical signal being indicative of sugar level in an ocular fluid. An ophthalmic sensor of the present invention is suitable for continuously monitoring of glucose concentration in a body fluid in a non-invasive or minimally invasive manner.

BACKGROUND OF THE INVENTION

Diabetes is a serious, lifelong disease which can cause long-term complications that affect almost every part of the body. This disease often leads to blindness, heart and blood vessel disease, strokes, kidney failure, amputations, and nerve damage. Uncontrolled diabetes can complicate pregnancy, and birth defects are more common in babies born to women with diabetes. Diabetes is widely recognized as one of the leading causes of death and disability in the United States.

One important aspect in the treatment of diabetes is the tight control of blood glucose levels, which requires frequent monitoring of blood glucose levels of patients so as to manage food intake and the dosage and timing of insulin injection. Tests for determining serum glucose concentration typically require blood collection. Blood collection is an invasive technique requiring arterial or venous puncture. A patient has to endure discomfort associated with needles or other devices to obtain blood samples for testing. Currently, millions of diabetics are forced to draw blood daily to determine their blood sugar levels. In addition, blood collection sometimes can be associated with problems in various ethnic settings. To alleviate the constant discomfort and inconvenience for these individuals, substantial effort has been expanded in the search for a non-invasive or minimally invasive technology to accurately determine blood glucose levels, in particular for a non-invasive or minimally invasive to continuously or at least frequently monitor blood glucose levels.

In recent years, various non-invasive and minimally-invasive technologies have been proposed in the academic and patent literature to monitor blood glucose levels by determining glucose concentrations in an ocular fluid, such as tears, aqueous humor, or interstitial fluid. For example, PCT International Publication WO 01/13783, discloses that an ophthalmic lens comprising a chemical sensor can be used to determine the amount of an analyte (e.g., glucose) in an ocular fluid, which is accessible to light. Such chemical sensors comprise a receptor specific for an analyte of interest and a detectable label (e.g., a

invention are disclosed in or are obvious from the following detailed description. It is to be understood by one of ordinary skill in the art that the present discussion is a description of exemplary embodiments only, and is not intended as limiting the broader aspects of the present invention.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Generally, the nomenclature used herein and the laboratory procedures are well known and commonly employed in the art. Conventional methods are used for these procedures, such as those provided in the art and various general references. Where a term is provided in the singular, the inventors also contemplate the plural of that term. As employed throughout the disclosure, the following terms, unless otherwise indicated, shall be understood to have the following meanings.

"Biocompatible", as used herein, refers to a material or a surface of a material or an article which does not deteriorate appreciably and does not induce a significant immune response or deleterious tissue reaction, e.g., toxic reaction or significant irritation, over time when implanted into or placed adjacent to the biological tissue of a subject. Preferably, a biocompatible material does not deteriorate and does not cause immune response or deleterious tissue reaction over at least 6 months, more preferably at least 1 year, most preferably at least 10 years. Exemplary biocompatible materials that are particularly suitable for producing a biocompatible sensor of the present invention are discussed below.

An "ophthalmic device", as used herein, refers to a contact lens (hard or soft), a corneal onlay, implantable ophthalmic devices used in, on or about the eye or ocular vicinity.

An "implantable ophthalmic device", as used herein, refers to an ophthalmic device which is used in, on or about the eye or ocular vicinity. Exemplary implantable ophthalmic devices include, without limitation, an intraocular lens, a subconjunctival lens, an intracorneal lens, and a shunt or implant (e.g., a stent, or a glaucoma shunt or the like) that can rest in the cul de sac of an eye.

The term "contact lens" employed herein in a broad sense and is intended to encompass any hard or soft lens used on the eye or ocular vicinity for vision correction, diagnosis, sample collection, drug delivery, wound healing, cosmetic appearance (e.g., eye color modification), or other ophthalmic applications.

"Ophthalmically compatible", as used herein, refers to a material or surface of a material which may be in intimate contact with the ocular environment for an extended period of time without significantly damaging the ocular environment and without significant user

"Molecular weight" of a polymeric material (including monomeric or macromeric materials), as used herein, refers to the number-average molecular weight unless otherwise specifically noted or unless testing conditions indicate otherwise.

A "polymer" means a material formed by polymerizing one or more monomers.

A "prepolymer" refers to a starting polymer which can be polymerized and/or crosslinked upon actinic radiation to obtain a crosslinked polymer having a molecular weight much higher than the starting polymer.

A "photoinitiator" refers to a substance that can initiate free radical polymerization and/or crosslinking by the use of light. Suitable photoinitiators are benzoin methyl ether, diethoxyacetophenone, a benzoylphosphine oxide, 1-hydroxycyclohexyl phenyl ketone and Darocur and Irgacur types, preferably Darocur 1173® and Darocur 2959®. Examples of benzoylphosphine initiators include 2,4,6-trimethylbenzoyldiphenylo-phosphine oxide; bis-(2,6-dichlorobenzoyl)-4-N-propylphenylphosphine oxide; and bis-(2,6-dichlorobenzoyl)-4-N-butylphenylphosphine oxide. Reactive photoinitiators which can be incorporated, for example, into a macromer or can be used as a special monomer are also suitable. Examples of reactive photoinitiators are those disclosed in EP 632 329, herein incorporated by reference in its entirety. The polymerization can then be triggered off by actinic radiation, for example light, in particular UV light of a suitable wavelength. The spectral requirements can be controlled accordingly, if appropriate, by addition of suitable photosensitizers.

A "visibility tinting agent" refers to a substance that dyes (or colors) a contact lens to enable a user to easily locate a contact lens in a clear solution within a lens storage, disinfecting or cleaning container. It is well known in the art that a dye and/or a pigment can be used as a visibility tinting agent.

A "dye" means a substance that is soluble in a solvent and that is used to impart color. Dyes are typically translucent and absorb but do not scatter light. Any suitable biocompatible dye can be used in the present invention.

A "Pigment" means a powdered substance that is suspended in a liquid in which it is insoluble. A pigment can be a fluorescent pigment, phosphorescent pigment, pearlescent pigment, or conventional pigment. While any suitable pigment may be employed, it is presently preferred that the pigment be heat resistant, non-toxic and insoluble in aqueous solutions. Examples of preferred pigments include (C.I. is the color index no.), without limitation, for a blue color, phthalocyanine blue (pigment blue 15:3, C.I. 74160), cobalt blue (pigment blue 36, C.I. 77343), Toner cyan BG (Clariant), Permajet blue B2G (Clariant); for a green color, phthalocyanine green (Pigment green 7, C.I. 74260) and chromium sesquioxide;

The term "molecular sensing moiety" employed herein in a broad sense and is intended to encompass, for example, a chemical or biochemical molecule or fragments thereof that is capable of interacting or reacting specifically with an analyte of interest in a sample to provide one or more optical signal. Exemplary molecular sensing moieties includes without limitation derivatives of phenyl boronic acid (for interacting with glucose), a receptor for specifically binding an analyte of interest, and an enzyme which reacts specifically with an analyte of interest.

Naturally, boronic acid compounds have been used for the synthesis of glucose sensors. Boronic acids are weak Lewis Acids composed of an electron deficient boron atom and two hydroxyl groups, which can interact with strong bases like OH- to from the anionic borate form, showing typically high pKa around 9 (Karnati, et al., A glucose-selective fluorescence sensor based on boronic acid-diol recognition, Bioorganic and Medicinal Chemistry Letters, 12, (2002), 3373-3377; Dicesare and Lakowicz, Charge transfer fluorescent probes using boronic acids for monosaccharide signaling, J. Biomedical Optics, 7(4), (2002), 538-545, incorporated herein by reference in their entirety). Boronic acids couple with diols to form a boronic acid diester group. The diol is linked covalently, and the reaction is fast and completely reversible. In comparison to the boronic acid group, the boronic acid ester group shows higher acidity (pKa ~ 6) due to a higher electrophilic boron atom. The phenylboronic acid group shows higher affinity for D-fructose with a smaller affinity for D-glucose, with binding constants of ~ 0.5 and 10 mM respectively (Dicesare and Lakowicz, Charge transfer fluorescent probes using boronic acids for monosaccharide signaling, J. Biomedical Optics, 7(4), (2002), 538-545, incorporated herein by reference in its entirety). The use of the boronic acid groups for sensing sugars is strongly dependent on the molecular geometry and the aromatic species where the boronic acid group is present, hence glucose sensitive probes can be made with a variety of affinities, in the mM range for blood glucose, and in the μM range for tear glucose.

Examples of optical signals include changes in the optical properties, including, but not limited to, a change in color, changes in intensity (absorbance or fluorescence) at different wavelengths, a spectral (absorption or emission) shift, changes in lifetime of luminescence (fluorescence, phosphorescence, and the like), and the like. A change in color can be observed by naked eyes and can be used in qualitative or semi-quantitative assays.

The term "receptor" employed herein in a broad sense and is intended to encompass, for example, a protein or fragment thereof or a biochemical compound that is capable of binding an analyte of interest in a sample. Exemplary receptors include, without limitation,

6,303,687 (incorporated by reference in their entireties); a water-soluble vinyl group-terminated polyurethane which is obtained by reacting an isocyanate-capped polyurethane with an ethylenically unsaturated amine (primary or secondary amine) or an ethylenically unsaturated monohydroxy compound, wherein the isocyanate-capped polyurethane can be a copolymerization product of at least one polyalkylene glycol, a compound containing at least 2 hydroxyl groups, and at least one compound with two or more isocyanate groups; derivatives of a polyvinyl alcohol, polyethyleneimine or polyvinylamine, which are disclosed in US 5,849,841 (incorporated by reference in its entirety); a water-soluble crosslinkable polyurea prepolymer described in US Patent No. 6,479,587 (herein incorporated by reference in its entirety); crosslinkable polyacrylamide; crosslinkable statistical copolymers of vinyl lactam, MMA and a comonomer, which are disclosed in EP 655,470 and US 5,712,356; crosslinkable copolymers of vinyl lactam, vinyl acetate and vinyl alcohol, which are disclosed in EP 712,867 and US 5,665,840; polyether-polyester copolymers with crosslinkable side chains which are disclosed in EP 932,635; branched polyalkylene glycol-urethane prepolymers disclosed in EP 958,315 and US 6,165,408; polyalkylene glycol-tetra(meth)acrylate prepolymers disclosed in EP 961,941 and US 6,221,303; and crosslinkable polyallylamine gluconolactone prepolymers disclosed in PCT patent application WO 2000/31150.

An ophthalmic lens of the invention may be produced by any convenient manufacturing means, including, for example, a computer-controllable manufacturing device, molding or the like. A "computer controllable manufacturing device" refers to a device that can be controlled by a computer system and that is capable of producing directly an ophthalmic lens or optical tools for producing an ophthalmic lens. Any known, suitable computer controllable manufacturing device can be used in the invention. Exemplary computer controllable manufacturing devices includes, but are not limited to, lathes, grinding and milling machines, molding equipment, and lasers.

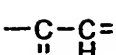
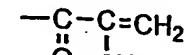
Preferably, contact lenses can be manufactured economically in large numbers by a conventional full-mold process involving disposable molds, the examples of which are disclosed in, for example, PCT patent application no. WO/87/04390 or in EP-A 0 367 513 (herein incorporated by reference in their entireties). More preferably, contact lenses can be manufactured economically in large numbers by a process described in European Patent EP 0 637 490 B1 (herein incorporated by reference in its entirety). In the process of EP 0 637 490 B1, a lens-forming material (e.g., a prepolymer solution) is introduced into a mold consisting of two mold halves, the two mold halves not touching each other but having a thin

first functionalizing the surface of a preformed ophthalmic device to obtain function groups and then covalently attaching a layer of a molecular sensing moiety. Surface modification (or functionalization) of an ophthalmic device is well known to a person skilled in the art. Any known suitable method can be used.

For example, the surface modification of a contact lens includes, without limitation, the grafting of monomers or macromers onto polymers to make the lens biocompatible, wherein monomers or macromers contain functional groups, for example, such as hydroxyl group, amine group, amide group, sulfhydryl group, –COOR (R and R' are hydrogen or C_1 to C_8 alkyl groups), halide (chloride, bromide, iodide), acyl chloride, isothiocyanate, isocyanate, monochlorotriazine, dichlorotriazine, mono- or di-halogen substituted pyridine, mono- or di-halogen substituted diazine, phosphoramidite, maleimide, aziridine, sulfonyl halide, hydroxysuccinimide ester, hydroxysulfosuccinimide ester, imido ester, hydrazine, axidonitrophenyl group, azide, 3-(2-pyridyl dithio)proprionamide, glyoxal, aldehyde, epoxy. The above mentioned functional groups can alternatively be introduced onto the surface of a contact lens by using other surface modification techniques, such as, for example, a surface treatment by energy (e.g., a plasma, a static electrical charge, irradiation, or other energy source), chemical treatments, or layer-by-layer deposition of polyelectrolytes (LbL coating).

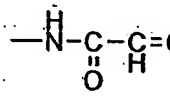
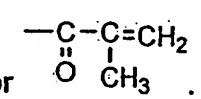
It is well known in the art that a pair of matching functional groups can form a covalent bond or linkage under known reaction conditions, such as, oxidation-reduction conditions, dehydration condensation conditions, addition conditions, substitution (or displacement) conditions, 2+2 cyclo-addition conditions, Diels-Alder reaction conditions, ROMP (Ring Opening Metathesis Polymerization) conditions, vulcanization conditions, cationic crosslinking conditions, and epoxy hardening conditions. For example, an amino group is covalently bondable with aldehyde (Schiff base which is formed from aldehyde group and amino group may further be reduced); an hydroxyl group and an amino group are covalently bondable with carboxyl group; carboxyl group and a sulfo group are covalently bondable with hydroxyl group; or a mercapto group is covalently bondable with amino group.

Exemplary covalent bonds or linkage, which are formed between pairs of crosslinkable groups, include without limitation, ester, ether, acetal, ketal, vinyl ether, carbamate, urea, amine, amide, enamine, imine, oxime, amidine, iminoester, carbonate, orthoester, phosphonate, phosphinate, sulfonate, sulfinate, sulfide, sulfate, disulfide, sulfinamide, sulfonamide, thioester, aryl, silane, siloxane, heterocycles, thiocarbonate, thiocarbamate, and phosphonamide.

C_1-C_6 alkyl, and R_2 is a C_3-C_{25} radical terminated with  or  ; and

n is an integer from1 to 5.

It is understood that the phenyl ring of a derivative of phenyl boronic acid can be substituted with electron withdrawing groups such as fluorine or nitrate and that the position of the boronic acid may vary.

One preferred example of R' in formula (1) or (2) is  or  .

A derivative of phenyl boronic acid of formula (1) or (2) having an olefinically unsaturated, crosslinkable radical can be incorporated in a lens-forming material for making an ophthalmic sensor device of the invention. In this case, a molecular sensing moiety can be copolymerized with other polymerizable components in the lens-forming material and thereby become parts of or be anchored onto the polymer matrix of the ophthalmic device.

Where a derivative of phenyl boronic acid of formula (1) or (2) without any olefinically unsaturated, crosslinkable radical, it can be entrapped in the polymer matrix by incorporating it in a lens-forming material before curing.

A derivative of phenyl boronic acid of formula (1) or (2) can interact or react with a sugar, preferably glucose, to cause changes in fluorescence lifetime, changes in fluorescence intensity, and/or spectral shifts. Any known suitable methods for measuring fluorescence intensity, and spectral shifts can be used in the invention to determine glucose concentration in an ocular fluid.

The previous disclosure will enable one having ordinary skill in the art to practice the invention. In order to better enable the reader to understand specific embodiments and the advantages thereof, reference to the following examples is suggested.

Example 1

This example illustrates boronic acid containing fluorophores (BAFs) which employ different mechanisms to induce spectral changes in the presence of sugar, in particular excited-state charge transfer (CT). CT is a versatile mechanism that can be applied to a large number of fluorophores, where the boronic acid group and an electron donor group are present on the same fluorophore. Here, the BA group [-B(OH)2] acts as an electron withdrawing group. However, in the presence of sugar and at an appropriate pH, the boronic

Leaching of the probes from the contact lens polymer is observed using the sample holder, which contained ~ 1.5 cm^3 buffer, at 20° C. A Varian fluorometer measured the intensity change as a function of *time* to determine the percentage signal change, corresponding to dye leaching. It should be noted that with no sample present, no intensity fluctuations or drifts are observed, indicating stability of the fluorometer Xenon-arc source.

To determine the usefulness of BAFs with regard to tear glucose sensing in a contact lens, it is necessary to compare both solution and lens based measurements.

In Solution

Stilbene Derivatives. Two stilbene derivatives, 4'-Dimethylaminostilbene-4-boronic acid (DSTBA) and 4'-Cyanostilbene-4-boronic acid (CSTBA) are used. The dimethylamino group is an electron-donating group. Cyano group is an electron withdrawing group. These two probes demonstrating both reduced and increased CT respectively in the presence of sugar.

In the case of DSTBA in solution, it is found that the emission spectrum shows a hypsochromic shift of about 30 nm and an increase in fluorescence intensity as the concentration of fructose increases. These dramatic and useful changes can simply be explained by the loss of the electron withdrawing property of the boronic acid group following the formation of the anionic form.

In the case of CSTBA stilbene derivative having two electron withdrawing groups, in the presence of sugar a bathochromic shift, some 25 or so nm, and a decrease in the intensity at pH 8, is observed. Tese results are opposite to that observed for DSTBA. This change has been attributed to an excited CT state present for the anionic form of CSTBA, where no CT states are observed for the neutral form of the boronic acid group, suggesting that the anionic form of the boronic acid group can act as an electron donor group.

It is observed that for both stilbene probes they have higher affinities for D-Fructose and that the affinity decreases for *D-Galactose* and *D-* Glucose.

Polyene Derivative. In order to test the suitability of longer wavelength probes in the contact lens, a polyene derivative, DDPBBA, 1-(p-Boronophenyl)-4- (p-dimethylaminophenyl)buta-1,2-deine, which combined a dimethylamino group and a boronic acid group in the *para* positions of each of the phenyl groups. As observed for DSTBA, there are a blue shift in the emission and an increase in the emission intensity with increasing sugar concentrations.

Chalcone Derivatives. Chalcone derivatives, unlike the stilbenes and polyenes, have the advantage of much longer wavelength emission. This is particularly attractive as longer wavelength emission reduces the detection of any lens or eye autofluorescence as well as

Comparing the responses of the stilbene probes based on a simple intensity ratio measurement, it is interesting to see the much greater response for fructose for CSTBA in the lens as compared DSTBA, where notable changes in intensity occur at < 20 mM [fructose]. However the glucose response of DSTBA in the contact lens appears more promising for [glucose] < 10 mM, where a 10 % fluorescence intensity change is observed for ~ 10 mM glucose at pH 8.0.

Polyene Derivative. The spectral response of DDPBBA in the contact lens is also different to that observed in solution. A decrease in intensity is typically observed for increasing sugar concentration, and a slight blue shift is evident for fructose binding. This is in contrast to solution-based responses which show both a blue shifted and increased emission. While the general spectral changes observed for both DSTBA and DDPBBA, are similar, a greater dynamic response to sugar is observed for DSTBA as compared to DDPBBA. In addition, the response of DDPBBA towards both glucose and fructose are similar over the sugar concentration range studied, as compared to the significantly different responses observed for both sugars for DSTBA and CSTBA.

Chalcone Derivatives. The response of Chalc 1 and Chalc 2 doped contact lenses display similar responses to sugar, only their respective emission wavelengths differ. Chalc 1 shows an emission centered around 560 nm in the lens as compared to 580 nm in solution, while Chalc 2 shows an emission centered at ~ 630 nm as compared to 665 nm in solution. In contrast to the responses observed in solution, a reduction in fluorescence intensity is observed for both Chalc 1 and 2 doped contact lenses. Interestingly, the solution response for Chalc 2 towards 100 mM fructose at pH 8.0 produces an ~ 3 fold increase in fluorescence emission, as compared to the ~ 2.6 fold reduction for the same fructose concentration in the contact lens.

Probe Leaching from the Contact Lens

To ascertain the practical use of a glucose sensing contact lens, leaching studies of the probes from within the lens are undertaken. Due to the very low concentration of probes within the lens, absorbance measurements could not be used to track the amount of unleached dye. Subsequently, we tracked the % loss of fluorescence emission from the lens as a function of time. While it could be argued that this method is problematic, for example, dye could have a different quantum yield inside and outside the lens, this method is used to simply give an indication of how long we needed to pre-leach the lenses before use, as well as to provide general information on the dye-lens compatibility.

methanol solution (pH 8.0, 2:1 *v/v)* for 1 hr, then rinsed extensively with Millipore water. The estimated value of *11-1/3 is* ~ 1.28, indicating the polarity within the lens is not indifferent than that of methanol (111 *13* for MeOH = 1.33). In retrospect, this is not surprising given that the contact lens is PVA based.

By determining both the pH and polarity within the contact lens it is possible to rationale the different spectral responses observed as compared to solution. As the solution pH increases the emission spectrum of DDPBBA displays a large blue shift. These spectral changes induced by the pH are due to the formation of the anionic form of the boronic acid group. As the anionic boron ate species is formed, the boron group is no longer an electron-withdrawing group, resulting in the removal and/or perturbation of the charge transfer nature of the excited state. An important feature here is the change in acidity (electrophilicity) of the boron group between the uncomplexed and complexed forms. Indeed this acidity change is the driving force enabling the use of the boronic acid moiety for sugar sensing. At a lower pH (such as in the contact lens), the simple complexation of the boronic acid with sugar does not fully result in a perturbation of the fluorophore, hence DDPBBA is not suitable as a wavelength ratiometric probe in the contact lens. The same however would be true at a much higher pH also. To induce a spectral change of the fluorophore, the complexation of the BAFs with sugar should result in a perturbation of the electronic properties of the fluorophore, i.e. from the neutral to the anionic form. It is believed that these BAFs typically display pKa around 9, with a *pKa* ~ 6 for the sugar complexed form. Hence these probes are ideal for solution sugar sensing in the pH range 6.5-8.5, which for blood glucose levels is ideal, where the maximum spectral change is usually observed in the pH range 7 -7.5. However, the low pH nature of the contact lens limits the spectral changes and therefore the dynamic range for tear glucose sensing. In addition these probes are polarity sensitive. For DSTBA and DDPBBA, as the polarity of the solvent increases, a red-shifted emission band can be observed, which accounts for the emission maximum difference between DDPBBA in the contact lens and in pH 6 solution. Similar rationale can also be drawn for the other BAFs considered here.

Although various embodiments of the invention have been described using specific terms, devices, and methods, such description is for illustrative purposes only. The words used are words of description rather than of limitation. It is to be understood that changes and variations may be made by those skilled in the art without departing from the spirit or scope of the present invention, which is set forth in the following claims. In addition, it should